24 23
~~32.~~ A method according to claim ~~31~~ wherein said target nucleotide sequence comprises a sequence of more than 8 nucleotides.--

Cl 4 cond'd

REMARKS

Claims 1-26 are pending and will remain so, and new claims 27-32 have been added by the present amendment, leaving claims 1-32 pending after entry of the present amendment. For the convenience of the Examiner in reviewing the amendment, a complete set of "clean" claims as they would appear after entry of the present amendment (with bracketed matter deleted and underlined matter insert), grouped according to subject matter first and then numbering, has been appended to this response.

The amendment

The specification has been amended to indicate that the present application is a continuation-in-part of the cross-referenced parent application, which was copending at the time of filing the present case, and hence also a continuation-in-part of the cited grandparent and great grandparent applications of which the parent was a continuation-in-part.

The claims have been amended to point out more clearly that which applicant considers to be his invention. Thus, Claim 9, the main claim in the group previously elected for prosecution, has been simplified and otherwise amended to point out that the invention now claimed is simply a method for producing a nuclease having the specified characteristics, i.e., when produced in a cell comprising a target nucleic acid which comprises a target nucleotide sequence, this nuclease specifically binds to the target nucleotide sequence and cleaves the target nucleic acid that is specifically bound to the nuclease. Support for this claim language may be found in the original claims as filed and throughout the specification,

as applicant believes this to be merely a reasonably conventional definition of what is commonly called a restriction nuclease.

The method of producing a nuclease of claim 9, as amended, comprises two simple steps, the first of which is: a) providing a preparation of a first polynucleotide encoding the nuclease. Claim 9 further specifies that this preparation does not comprise a polynucleotide encoding an enzyme which will be recognized as what is commonly called a restriction nuclease-related modification enzyme (i.e., in the terminology of claim 9, an enzyme which protects the target nucleic acid from the nuclease by specifically binding to the target nucleotide sequence and enzymatically modifying the target nucleic acid such that the target nucleic acid is not cleaved by the nuclease). Again, the amendment is supported throughout the instant specification, including, for instance, page 7, lines 21-25, and the examples which describe production of hybrid restriction enzymes for which no corresponding modification enzyme is available.

The second step of the method of producing a nuclease in claim 9 is: b) delivering the preparation of the polynucleotide encoding the nuclease into the cell under conditions such that this polynucleotide expresses the nuclease, thereby producing the nuclease, as recited in the claim preamble. This language is also believed to be supported throughout the specification, including the examples, and in the original claims.

Instant claims 10-17 and 19-26, which depend from claim 9, have been amended to correct minor spelling and grammatical errors, and to maintain proper antecedent reference by conforming the language to that of amended claim 9. Therefore, these changes are also supported by the specification in the manner of corresponding amendments to claim 9.

Applicant has also added new claim 27, depending from claim 9, which is directed to an embodiment of the invention method previously described by claim 9 as originally filed.

Thus, claim 27 recites a method of claim 9 where the cell in which the nuclease is to be produced comprises the target nucleic acid and, further in this method, the target nucleic acid is to be specifically inactivated. Claim 27 further specifies that the nuclease produced in the cell (from the polynucleotide added in claim 9) specifically inactivates the target nucleic acid by specifically binding to the target nucleotide sequence and cleaving the target nucleic acid bound to the nuclease (cf. original claim 9 reciting a method for enzymatically inactivating a target DNA using a gene for a nuclease which specifically recognizes and inactivates the target.)

New claim 28, depending from claim 20 and, ultimately, from claim 9, has been added to claim a preferred embodiment in which the catalytic domain (of a hybrid endonuclease) is obtained from the *FokI* restriction endonuclease, as exemplified in the working examples of the present application. Similarly, new claim 29 is directed to a preferred embodiment of the method of claim 9 wherein the recognition domain (of a hybrid nuclease) comprises a zinc finger domain, as disclosed throughout the specification New claim 30 describes a method according to claim 9 wherein the target nucleic acid is a DNA:RNA hybrid. This embodiment is supported, for instance, at page 46, lines 3-21, and in the preceding description of experimental results beginning at page 40, line 8.

New claims 31 and 32, which depend from claim 9, are directed to preferred embodiments in which the nuclease produced binds to a target nucleotide sequence that comprises a sequence of more than 6 nucleotides (claim 31) or, more than 8 nucleotides (claim 32). Support for claims 30 and 31 may be found, for instance, at page 3, lines 1-20, of the instant specification.

In addition to the claims originally depending from claim 9, applicant has amended claim 1 so that it now depends from amended claim 9, and further to simplify the language and to reflect the changes in claim 9, while still describing a cloning method using a ligase gene, as in original claim 1 as filed. Thus, amended claim 1 now recites a method of producing a nuclease, as in claim 9, where the cell in which the nuclease is to be produced is to be cloned. Further, that cell comprises the target nucleic acid and that target nucleic acid is required for cloning of the cell. Besides the two steps of amended claim 9, the method of amended claim 1 further comprises the steps of c) providing a third polynucleotide encoding a DNA ligase; and d) delivering that third polynucleotide to the cell before or concurrently with the step (in claim 9) of delivering the preparation of the first polynucleotide into the cell. This polynucleotide encoding DNA ligase is delivered to the cell, of course, under conditions such that the DNA ligase is produced, whereby the target nucleic acid is protected by the ligase from inactivation by the nuclease produced in the cell. Finally, the method of claim 1 as amended calls for step e) cloning the cell producing the nuclease and ligase, as recited in the preamble of amended claim 1. These amendments of claim 1 are supported throughout the instant specification, including, for instance, page 8, lines 6-22, and the examples which describe cloning of hybrid restriction enzymes using a cell also transformed with a ligase gene. The claims depending from claim 1 (claims 2-8) also have been amended to conform the language to amended claims 1 and 9 from which these claims now depend, as described above for corresponding claims depending directly from amended claim 9, with similar support provided.

Since no new matter is believed to be introduced by the present changes, entry on the record and consideration of the instant amendment is believed proper and is respectfully requested.

Request for priority of parent applications

For purposes of establishing priority of claim 9 as presently amended, applicant first notes that the present application was filed December 20, 1995, which was during the copendency of the parent application, of which the present application has been designated a continuation-in-part (CIP), Serial No. 08/346,293, filed November 23, 1994, issued as U.S. Patent No. 5,487,994 on January 30, 1996. Further, this parent application was a CIP of the grandparent application, Serial No. 07/126,564, filed September 27, 1993, issued as U.S. Patent No. 5,436,150, July 25, 1995. In turn, the grandparent application was a CIP of the great grandparent, copending Serial No. 08/017,493, filed February 12, 1993, abandoned April 18, 1994, which was a CIP of the great great grandparent, having the same title as the grandparent, Serial No. 07/862,831, filed April 3, 1992, issued as U.S. Patent No. 5,356,802, on October 18, 1994. Accordingly, the present case is the most recent in the chain of applications above which meet the copendency requirement for the present claims to obtain benefit of the priority date of the earliest filed application. In addition, each of these applications was filed by applicant as sole inventor and, therefore, the requirement for a common inventor for benefit of the priority date also is met for this chain of copending applications.

As to support for present claim 9, as amended, applicants first note that the great great grandparent application, Serial No. 07/862,831, entitled "Functional Domains in Flavobacterium Okeanokoities (FOKI) Restriction Endonuclease," discloses at least one embodiment of the currently claimed method for producing a nuclease of claim 9, for instance, in the Detailed Description, wherein applicant teaches the production of hybrid restriction enzymes comprising the nuclease domain of the FokI enzyme linked to a

recognition domain of another enzyme or DNA binding protein. See U.S. Patent No. 5,356,802, col. 25, lines 25-43 (copy filed herewith). Applicant particularly notes that the methods of production of hybrid enzymes taught in this great grandparent application necessarily would be conducted without a polynucleotide encoding a corresponding modification enzyme, as specified in claim 9 as presently amended, since no such enzyme existed for the hybrid enzymes disclosed. Further, the method of producing a nuclease of claim 9 embodied in the '802 patent also does not involve transforming the host with a DNA ligase gene as specified in the process of instant claim 1. Accordingly, applicant believes that instant claim 9, as amended, is supported by the great great grandparent disclosure as required for benefit of the filing date of that disclosure.

The great grandparent, copending Serial No. 08/017,493, abandoned in favor of the grandparent, contains at least the disclosure of the great great grand parent and therefore provides support for present amended claim 9 in like manner. The grandparent application, Serial No. 07/126,564, entitled "Functional Domains in Flavobacterium Okeanokoities (FOKI) Restriction Endonuclease," further discloses at least one embodiment of the method of claim 9, for instance, in working Example XV which describes initial cloning of a hybrid enzyme comprising the Ubx homeobox recognition domain, also necessarily without a corresponding modification enzyme, since no such enzyme existed. See U.S. Patent No. 5,436,150, col. 22, line 40-col. 25, particularly col. 22, lines 13-20 (copy filed herewith). As in the later disclosures, the method of producing a nuclease of claim 9 embodied in the '150 patent does not involve transforming the host with a DNA ligase gene as specified in the process of instant claim 1. Accordingly, applicant believes that instant claim 9, as amended, is properly supported for benefit of the priority date of the grandparent application.

Finally, the parent application, entitled "Insertion and Deletion Mutants of FokI Restriction Endonculease," Serial No. 08/346,293, expressly incorporates by reference the three parental applications listed above, and further discloses an embodiment of claim 9 in the disclosure of the hybrid restriction enzyme produced by linking the Ubx DNA binding protein domain to the cleavage domain of *FokI*, for instance at col. 1, lines 53-56, and col. 7, lines 58-61.

In view of the above support provided for amended claim 9 in each of the cited parental applications in the recited chain of copendency, and the substitute Declaration and Power of Attorney to be executed by applicant (unsigned copy filed herewith) and filed herewith, perfecting the priority claim by asserting benefit of each of the above parent applications, applicant respectfully requests that claim 9 as presently amended be granted benefit of the filing date of each of these parent applications. In particular, claim 9 as amended should be examined in view of the effective filing date of the great great parent application, Serial No. 07/862,831, filed April 3, 1992.

Treatment of the amended and new claims under the restriction requirement

Claims 1-26 are pending and have been subjected to a restriction requirement defining the inventions of Group I (claims 1-8; method of cloning restriction endonucleases) and Group II (claims 9-26; method for enzymatically inactivating a target DNA). Applicant traversed this restriction requirement, but made an election, as required, of Group II, in the response filed April 3, 1997. The present Office action maintains that this requirement was proper and has therefore made the requirement final and withdrawn claims 1-8 from consideration.

For the purpose of maintaining the right to petition the restriction requirement, applicant continues to traverse same on the grounds previously stated. However, applicant has now amended claim 9 to describe a generic method of producing a nuclease which encompasses both the elected method for enzymatically inactivating a target DNA (Group II) and the non-elected method of cloning restriction endonucleases. Thus, new claim 27, which depends from amended claim 9, is directed to a method of producing a nuclease wherein the cell comprises a target nucleic acid that is to be specifically inactivated by the nuclease, essentially as in the method previously described by claim 9 as filed. Accordingly, applicant believes that new claim 27, which effectively replaces original claim 9, may properly be designated as corresponding to the elected invention of Group II. Similarly, new claims 28 and 29, which depend from claim 9 via new claim 27, also should be designated as corresponding to elected Group II and examined therewith.

Applicant further believes that claim 9, since it encompasses new claim 27 which replaces claim 9, also may properly be designated as corresponding to the elected invention of Group II and examined with the current claims previously so designated. Similarly, new claims 30-32, which for convenience in limiting the number of dependent claims have been made to depend directly from amended claim 9, also can be readily seen as encompassing the method of claim 27, since the recited enzyme characteristics also are acceptable in the method of claim 27. Therefore, claims 30-32 also may properly be designated as corresponding to elected Group II and examined therewith.

In addition, applicant notes that claim 9, as amended, further encompasses the invention of Group I as well as that of Group II, as illustrated by amended claim 1 which recites a method of cloning a cell producing a nuclease using a ligase as in original claim 1. Hence, in accordance with 37 CFR § 1.141, which provides that more than one species of an

invention, not to exceed a reasonable number, may be specifically claimed in different claims in one national application, under the given conditions, applicant respectfully submits that independent claim 9 as amended is generic to all claimed species of this method of producing a nuclease and, therefore, amended claim 9 is a linking claim for the two claimed species of the invention (in dependent claims 1 and 27). See, MPEP § 8.09.03. Further in accordance with 37 CFR § 1.141, all the claims to species within this genus in excess of one are written in dependent form or otherwise include all the limitations of the generic claim (i.e., the claims to the species involving cloning using ligase, e.g., amended claim 1, and to the species involving inactivating the target nucleic acid (claim 27), as well as all other claims, now depend directly or indirectly from amended claim 9).

Applicant therefore respectfully requests that present linking claim 9 be examined with the invention elected previously, Group I, in accordance with MPEP § 8.09, and further, if this generic claim is subsequently found allowable, that applicant be advised of the allowable generic claim and that claims drawn to the non-elected invention of Group I (claims 1-8, as amended) are no longer withdrawn, since they are fully embraced by the allowable generic claim. MPEP § 8.09.02(c)(i). Since the claims of Group II describe the only other species of amended claim 9 presently claimed, applicants further respectfully request that, if linking claim 9 is found allowable, the Examiner thereafter examine the claims of Group II. MPEP § 8.09.04.

Informal Drawings

Applicant acknowledges the notification that the application has been filed with informal drawings under 37 CFR 1.85 and will provide formal drawings in due course, when the application is allowed.

Objections and rejection under 35 USC § 112, first paragraph

Claims 9-26 are rejected, and the specification is objected to, under 35 USC § 112, first paragraph, as the Office believes that the specification fails to teach how to make and/or use the claimed invention.

As far as applicant is able to understand, there are two grounds for the objection to the specification. First, the Examiner is concerned about two particular statements dealing with experimental results which the Examiner believes are contradictory. The Examiner concludes that "[o]ne of ordinary skill in the art reading this disclosure would not know what the results of the tests were and would not know how to practice the invention." Applicant does not concede that understanding the particular statements cited by the Examiner would be required to know how to practice the claimed invention. However, as shown below, the cited statements do not appear contradictory when properly considered in the context of the disclosure as a whole, as § 112 requires. Therefore, applicant believes that the disclosure would be sufficient for one of ordinary skill to understand the cited test results and, in any event, to practices the invention as now claimed in amended claim 9.

The basis for the Examiner's second concern about the disclosure is not entirely clear to applicant. Thus, the Examiner asserts that "[o]ther than a general stating of the subject matter of claims 9-28 in the specification, e.g. ..., there is no enablement in the instant specification that would teach the ordinary artisan reading this specification to practice the subject matter of the instant claims." Regarding this second basis for the rejection under § 112, applicant respectfully submits that the Office has failed to meet its burden of providing acceptable evidence or reasoning as required to support such a rejection.

As to the first of the above concerns about the present disclosure, applicant respectfully requests the Examiner to direct his attention to the context in the specification where the two supposedly contradictory statements are made. Thus, it is well established law that the specification as a whole must be considered in determining whether the scope of enablement provided by the specification is commensurate with the scope of the claims. *In re Moore*, 58 CCPA 1042, 1047, 439 F.2d 1232, 1235, 169 USPQ 236,238 (CCPA 1971), quoted with approval in *In re Johnson* 194 USPQ 187, 193 (1977).

The specification as a whole in this case includes disclosures of the invention concept, that the potential lethality of a nuclease gene can be circumvented for cloning such a gene by expression of ligase from a ligase gene added to the host cell, which presumably repairs damage to cellular DNA caused by the nuclease. See, for instance., page 19, lines 12-16 and page 36, lines 17-25. The pertinent context of the first statement cited in the Office action further includes Figure 8, referenced in that first statement, which is a photographic of transformed cells recovered from the tests referenced in the two cited statements. In addition, the context of the two statements includes at least the sentence immediately preceding the first cited statement, where the specification recites:

> ...The pTZ19R that does not carry a hybrid endonuclease gene was used as a standard control to compare the efficiency of transformation of the competent cells. **BL21 (DE3) (pACYC *lig*) transformed at about 5-10 fold lower efficiency as compared to the BL21(DE3) cells (Figure 8).**

Specification page 34, line 24-page 35, line 3 (**boldface type** added here to identify the first cited statement).

As a preliminary matter, applicant notes that the genetic nomenclature in the disclosure is believed to be entirely conventional in the art and presumably, therefore, this is not the basis for the Examiner's belief that "[o]ne of ordinary skill in the art reading this

disclosure would not know what the results of the tests were...." For instance, properly considering the above disclosure as a whole, including Figure 8 and the sentence preceding the first cited statement, applicant believes a skilled worker familiar with genetic nomenclature would simply take this disclosure to mean that: (1) the cloning plasmid [pTZ19R] was introduced into host cells [BL21(DE3)] containing or lacking a plasmid carrying a ligase gene (pACYC *lig*); and (2) as the photograph of transformed colonies illustrates, the results of this first test were that the number of transformed colonies obtained with this cloning plasmid was 5-10 fold lower for cells with the ligase gene (Fig. 8, col. C, bottom row) than for cells without it (C, top). Thus, applicant believes the results of the test referenced in the first cited statement would be clear to one of ordinary skill when, as required, the specification as a whole is considered.

The specification then immediately sets the context for the second cited statement, with a section title and one additional preceding sentence citing Table II, as follows:

> 6. TRANSFORMATION EFFICIENCY OF HYBRID GENES
>
> The transformation efficiency of two different endonuclease genes, pET-15b:ZFHD1-F_N and pET-15b:ZF-QQR-F_N, into BL21 (DE3), with and without pACYC *lig* is summarized in Table II. **BL21 (DE3) with pACYC *lig* transform about 2-fold better compared to BL21 (DE3) without the pACYC *lig*.**

Specification page 35, lines 3-10 (**boldface type** added here to identify second cited statement). As readily seen by replacing the conventional genetic nomenclature with corresponding plain language, the above disclosure simply states (1) the object of the second transformation tests was comparing "[t]he transformation efficiency of two different endonuclease genes ... into [the same host cells as in the first test], with and without [the ligase gene]," and (2) the directly observed results in this second test, set forth in Table II and illustrated in Figure 8, were that about 2 fold more transformed colonies were obtained with the plasmids carrying nuclease genes from cells with the ligase gene (Fig. 8 A and B, bottom)

than from cells without ligase (A and B, top). Hence, applicant believes the results of the test referenced by the second cited statement also would be clear to one of ordinary skill, considering the specification as a whole.

Finally, the pertinent context of the two cited statements includes the following conclusion, immediately after the second cited statement, tying together the results of the above two tests, as follows:

> Taking into consideration 5-10 fold lower efficiency of BL21 (DE3) (pACYC *lig*) as compared to BL21 (DE3), this translates into about 10-20 fold difference between *E. coli* strains with and without pACYC *lig*.

Specification page 35, lines 3-10. Applicant believes that one of ordinary skill in the art also would readily understand from the overall disclosure the significance of "[t]aking into consideration 5-10 fold lower efficiency" of the ligase containing cells as determined in the first test. Thus, the data in Table II indicate that more nuclease gene-transformed colonies were recovered from cells with ligase than from cells without ligase, in accord with the present invention. However, the results of the colony counts in Table II indicated about 2 fold more nuclease transformants with ligase than without, which, considered alone, might mean that the nuclease gene killed host cells without ligase only about twice as often as cell with ligase. However, the photograph of transformants with cloning plasmid alone shows that, without the nuclease gene, far less transformants were obtained from cells with (Fig. 8 C, top) ligase than without (C, bottom). It is therefore apparent that putting the nuclease gene into the cloning vector in cells with ligase (A or B, top) had little effect on the yield of transformants obtained with cloning plasmid alone (C, top). In contrast, however, cells without ligase produce a much larger crop of transformants with plasmid alone (C, bottom), but these were grossly decimated by putting the nuclease gene into the plasmid (A or B).

Thus, Figure 8 provides stark visual evidence that the difference in nuclease killing between host cells with and without ligase was far more than the two fold effect implied from the colony counts of nuclease transformants alone, as in Table II. Further, the error in considering only the nuclease transformants is also immediately apparent from Figure 8—with the cloning plasmid alone, host cells with ligase (C, top) happen to yield many fewer transformants than those without (C, bottom). Applicant believes this phenomenon is well known, that the yield of transformants (i.e., the "efficiency of transformation") differs from one host cell line to another, for various reasons having nothing to do with the nature of the transforming gene.

In summary, Figure 8 dramatically illustrates what the specification as a whole discloses in greater detail, that the first test above indicates that cells with ligase had a 5-10 lower transformation efficiency with control plasmid alone and, therefore, that the 2-fold better recovery of nuclease gene transformants with ligase in the second test was multiplied by the 5-10 fold lower transformation efficiency of those cells, to obtain a proper quantitative estimate of the dramatic (10-20 fold) difference in killing by the nuclease gene of host cells with and without. Accordingly, applicant respectfully submits that one of ordinary skill would understand from the above disclosure as a whole, not only what the observed results of each of the two disclosed tests were, but also that the two cited statements are not contradictory because they refer to two different tests which are both needed to determine the quantitative effect of ligase on nuclease gene cloning, according to the invention.

What else the cited statements might mean to one skilled in the art, properly considering the specification as a whole, the present rejection does not say and applicant therefore has no clue. If the Examiner is aware of any facts which support the proposition that "[o]ne of ordinary skill in the art reading this disclosure would not know what the results of

the tests were and would not know how to practice the invention," applicant respectfully requests a declaration with regard to these facts under 37 CFR 1.107(b). Otherwise, this objection appears to be based on a misplaced concern that the two statements out of context may appear contradictory and, therefore, withdrawal of this basis for rejection under the first paragraph of § 112 is believed to be proper and is hereby respectfully requested.

Turning now to the second concern about the disclosure underlying the present objections and rejection under § 112, applicant would first respectfully remind the Examiner of well settled law in this area. For instance, the Office bears the burden of establishing a lack of enablement under § 112. *In re Hogan*, 194 USPQ 527, 539 (CCPA 1977). Further, even when "unpredictability" in a field may create reasonable doubt as to the accuracy of a broad statement supporting enablement, and even when the statement is, on its face, contrary to generally accepted scientific principles, the Court of Customs and Patent Appeals (predecessor to the U.S. Court of Appeals for the Federal Circuit), has clearly articulated that

> [I]t is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with a contested statement.

In re Marzocchi, 169 USPQ 367, 369 (CCPA 1967). To support of rejection under § 112, the evidentiary standard is the same as throughout ex parte prosecution, namely, a preponderance of the evidence. *In re Oetiker*, 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). A preponderance of the evidence exists when it suggests that it is more likely than not that the assertion in question is true. *Herman v. Huddleston*, 459 U.S. 375, 390 (1983).

In the present case, applicant respectfully submits that the Office has failed to meet its initial burden of supporting the conclusion, that the disclosure fails to enable the full scope of the claims, with any acceptable reasoning or evidence, much less by a preponderance of

evidence. Applicant therefore believes that the instant rejection for lack of enablement is unwarranted and may properly be withdrawn.

In formulating the present rejection, the Examiner first asserts that "[o]ther than a general stating of the subject matter of claims 9-28 in the specification, e.g. ..., there is no enablement in the instant specification that would teach the ordinary artisan reading this specification to practice the subject matter of the instant claims." The Examiner then makes the general observation that "[m]ost of the specification is devoted to a method of cloning using a ligase, which is the subject of claims 1-8 that were non-elected," evidently implying that the quantity of disclosure on the elected invention should be dispositive of the enablement issue. The Examiner also acknowledges that elsewhere in the instant Office Action "it is maintained that it would have been obvious to practice the method of [elected] claims 9-26," thereby tacitly acknowledging that practicing the invention would not be beyond the capabilities of one of ordinary skill. But, the Examiner still generally maintains that "the specification does not teach this [invention]."

Applicant is unable to discern in the above general concerns any acceptable evidence or reasoning contradicting applicant's assertion that the disclosure would enable a skilled worker to practice the full scope of the invention as claimed, as required under *Marzocchi, supra.* As to the implication that the quantity of disclosure is not sufficient to enable the elected invention, applicant further notes that the law requires a rejection for lack of enablement to be based on the evidence as a whole, and does not require working examples illustrating each aspect of the invention, as stated, for instance, in the following portion of the *Marzocchi* opinion:

> The first paragraph of § 112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance.

In re Marzocchi, 169 USPQ 367, 369 (CCPA 1967). It is evident, therefore, that the above concerns about "a general stating of the subject matter" and "[m]ost of the specification is devoted to [a non-elected invention]" have no bearing on a legal determination of whether the disclosure enables the full scope of the claims.

The only specific criticism of the disclosure raised by the Examiner appears to be that, "[p]resuming for the sake of argument that there might be some problems, e.g. in placing a gene coding for a nuclease from a bacteria into a plant or animal cell and having it express, this is not addressed in the instant specification." Absent objective evidence supporting the concern about expressing bacterial nuclease genes in plant or animal cells, this reasoning appears to represent only the opinion of the Examiner, and a highly speculative opinion, also. If the Examiner is aware of any facts which support this concern with respect to a particular nuclease which might be used in the invention method, applicant respectfully requests a declaration with regard to these facts under 37 CFR 1.107(b).

This speculation about difficulties with nuclease gene expression seems to indicate a concern that the scope of the present claims may include some combinations of sequence-specific nuclease and host cell which are inoperative. Applicants believe this concern is misplaced because enablement of the present claims under § 112 does not require that every existing sequence-specific nuclease work equally well, or even at all, in the claimed method, as illustrated, for instance, by the Patent Board's decision in *Ex parte Mark*, 12 USPQ 1904 (BPAI 1988). Claim 1 of *Mark* reads as follows:

> A synthetic mutein of a biologically active native protein in which the native protein has at least one cysteine residue that is free to form a disulfide link and is non-essential to said biological activity, said mutein having at least one of said cysteine residue substituted by another amino acid and said mutein exhibiting the biological activity of said native protein."

Mark had described only muteins of IFN-β, IL2 and TNF, and the Examiner had rejected the claim as overly broad. The Board reversed.

The Board in *Mark* emphasized that proteins which do not retain the biological activity of the native protein fell outside the scope of the claim. (So, here, do divalent metal salts that do not exhibit biological activity.) The Board further stated:

> The fact that a given protein may not be amenable for use in the present invention in that the cysteine residues are needed for the biological activity of the protein does not militate against a conclusion of enablement. One skilled in the art is clearly enabled to perform such work as is needed to determine whether the cysteine residues of a given protein are needed for retention of biological activities.

Similarly, one skilled in the art is clearly enabled to perform such work as is needed to determine whether a given sequence-specific nuclease may be effectively used in a given host cell and, hence, whether that nuclease and host cell combination falls within the scope of the claims. All the practitioner need do is introduce the nuclease gene into the selected host cell using conventional genetic technology known to be appropriate for that cell and then examine the effects of the nuclease on the target polynucleotide using an appropriate assay (a straightforward exercise, e.g., as described in the specification). This simple tests is not believed to involve any undue experimentation and, therefore, does not indicate a lack enablement of the full scope of the claims.

Further, practicing the invention of the present claims does not require the practitioner to survey all combinations of existing sequence-specific nucleases and hosts to find out which are suitable for the claimed method and which are not. This is true because the purpose of the claims is simply to provide a definition of the invention for a practitioner to use in determining whether any particular activity which (s)he desires to carry out would infringe the patent. Therefore, a skilled practitioner is simply required to select a combination of sequence-specific

nuclease and host cell in which the practitioner is presumably already interested, according to the invention, and test whether or not useful production of nuclease, according to claim 9 and, if so desired, inactivation of target nucleic acid (claim 27) or a nuclease producing clone (claim 1), are observed. If not, the selected combination of host and nuclease simply does not fall within the scope of the present claims.

Thus, the claims as presently proposed are of reasonable scope. They extend to a sequence-specific nuclease tightly defined by, *inter alia*, the requirement that, when produced in a cell comprising a target nucleic acid which comprises a target nucleotide sequence, it specifically binds to the target nucleotide sequence and cleaves the target nucleic acid; and sequence-specific nucleases are readily obtained as disclosed in the specification. The choice of nuclease and cell will be determined by the potential infringer's own interests.

In conclusion, applicant believes the disclosure has been shown to be adequate to enable the full scope of all the claims as presently amended and otherwise to meet all requirements of § 112 as well. Applicant also respectfully submits that the Office has not provided any acceptable reasoning or evidence to the contrary, as required, much less a preponderance of evidence. Accordingly, applicants believe that the present rejection of claims 9-26 under the first paragraph of § 112 may properly be withdrawn.

Rejections over the art

Claims 9, 12-14, 18 and 25-26 are rejected under 35 USC § 102(b) as being anticipated by Wilson (A). This document is said to teach the placing of a gene encoding a nuclease into a plasmid, delivering the plasmid into cells and inactivation of non-methylase clones by the endonuclease after expression.

Applicant first notes that claim 9, as presently amended, specifies that a preparation of a polynucleotide (e.g., a plasmid) encoding a nuclease is delivered into the cell under conditions under conditions which may be suitable for inactivation of the host cell (e.g., as now recited in new claim 27 depending from amended claim 9). However, claim 9 further expressly specifies that the preparation encoding the nuclease does not comprise a second polynucleotide encoding a "modification" enzyme corresponding to the encoded nuclease (i.e., an enzyme which protects the target nucleic acid from the nuclease by specifically binding to the target nucleotide sequence and enzymatically modifying the target nucleic acid such that the target nucleic acid is not cleaved by the nuclease). As disclosed in the Wilson patent, for instance, in the paragraph immediately before the cited portion, the described method of cloning restriction enzymes depends upon selection of methylase containing clones from libraries which also contain DNA fragments coding for the corresponding restriction genes. Col. 3, lines 53-59. In other words, Wilson discloses that all preparations of DNA libraries used for isolation of restriction endonuclease genes by delivery into cells also contained a polynucleotide encoding the corresponding modification (methylase) gene.

Therefore, claim 9 as amended expressly distinguishes over the method of producing a nuclease disclosed by Wilson, and the rejection of claims 9, 12-14, 18 and 25-26 under 35 USC § 102(b) over Wilson (A) may properly be withdrawn.

Claims 9-20 and 22-26 are rejected under 35 USC § 103 as being obvious over Wilson (A), cited as described above in the rejection for anticipation. The Office believes it would have been obvious to deliver the gene coding for the nuclease by liposomes, to integrate it into the chromosome, to use a hybrid or other "non-natural" restriction endonuclease or to place the gene into mammalian or plant cells, absent convincing [evidence] to the contrary. The Office asserts that "Essentially what applicant is claiming is

placing a gene encoding a nuclease into some cell, having the gene produce the nuclease and then having the nuclease 'inactivate' the DNA of the cell by digesting it." The Office further notes that claim 21 is not rejected because it is limited to hybrid restriction endonucleases which the examiner has been unable to find in the prior art.

Applicant again notes that claim 9, as amended (as well as all other claims, since they depend from claim 9), now specifies that the polynucleotide preparation encoding the nuclease does not contain a polynucleotide encoding a corresponding modification (methylase) enzyme. Further, applicant notes, as cited above, that the only method for producing a restriction nuclease taught in the cited portion of Wilson requires a library containing a gene for a corresponding modification (methylase) gene. Applicant also notes that the Background in Wilson discloses a method of cloning a restriction enzyme gene using phage, which also relies on a library containing a gene for the related modification enzyme. Wilson, col. 2, lines 22-34. Therefore, no method taught in the Wilson patent meets the description of present claim 9, at least with respect to the lack of a polynucleotide encoding a corresponding modification enzyme. Further, Wilson does not expressly suggest a method according to present claim 9, excluding a modification enzyme gene. Indeed, by emphasizing that the disclosed method is really a method for overproduction of restriction enzymes and their corresponding modification enzymes (e.g., leading off the Summary, col. 2, lines 45-58), applicant submits that the Wilson disclosure, fairly considered as a whole, actually teaches away from the method of claim 9, as presently amended, which precludes use of such a modification enzyme gene.

In view of the present amendment, therefore, applicant believes that amended claim 9, as well as all other claims, since they now depend from claim 9, are not suggested by Wilson

(A). Hence, the instant rejections of claims 9-20 and 22-26 under 35 USC § 103 as being obvious over Wilson (A) may also be withdrawn.

In view of the above, all pending claims, as amended, are submitted to be in condition for allowance and a Notice to that effect is requested. If Examiner Patterson has any questions about this matter which he believes may be resolved by discussion, he is sincerely encouraged to telephone the undersigned counsel at one of the numbers listed below.

Respectfully submitted,

CUSHMAN DARBY & CUSHMAN
Intellectual Property Group of
PILLSBURY MADISON & SUTRO LLP

by _____

PNK:PCK
1100 New York Avenue, N.W.
Ninth Floor
Washington, D. C. 20005-3918
Tel: (202) 861-3000

Paul C. Kimball
Reg. No. 34,610
Tel. (202) 861-3619
Fax: (202) 822-0944